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AMRHAI *News*

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Last year was a seminal year for antimicrobial resistance (AMR). It has reached international prominence as a key problem facing humanity. The UK has taken a leading role in achieving this, not least through championing by the Chief Medical Officer. The public voted for AMR as the 'grand challenge' for the Longitude Prize 2014, with contenders now required to develop novel diagnostics that promote fast, appropriate treatment of infected patients, and better stewardship of the antibiotics we have. Antibiotic stewardship was also a key message of the first ESPAUR report, which linked antibiotic prescribing in primary and secondary care in England with resistance rates, and allowed organizations to benchmark themselves against regional and national results for the first time; unsurprisingly, higher prescribing is generally associated with more resistance. Geographic variations in resistance were highlighted on a larger scale in the WHO's first *Global Report on Surveillance* of AMR; this drew disparate data together, identified gaps in our knowledge, and emphasized the importance of key resistances in select pathogens. The dwindling pipeline of new antibiotics active against such multi-resistant organisms was highlighted by the Prime Minister, who established an independent *Review on AMR* (where I spend some of my time) specifically to assess its economic impacts and to advise how drug discovery and development efforts can be invigorated globally. The *Review*'s first paper estimated that, left unchecked, AMR could cause 10 million deaths per year by 2050 and would have cumulative costs of US$100 trillion. These staggering projections have brought AMR to a much wider global audience. The challenge in moving forwards is to build on the momentum, and to promote the multidisciplinary relationships that will be needed to deliver solutions to the AMR problem. By collaborating widely with government, academia, industry, expert and other organizations both in the UK and overseas, AMRHAI will continue to play its part.

Neil Woodford

Carbapenem-resistant bacteria and endoscopes

There have been recent reports of transmission of carbapenem-resistant bacteria associated with endoscopes (see **example 1** and **example 2**). These reports refer specifically to duodenoscopes rather than endoscopes more generically. Duodenoscopes have an extra channel (the "raiser-bridge" channel), which carries only a wire, used to flip a metal lever out of the endoscope tip once in position such that accessories emerge at 90^{o} to cannulate the bile duct. In many duodenoscopes, the raiser-bridge channel is unsealed and can become contaminated. Decontamination is difficult as the lack of free space between the wire and the lumen makes it inaccessible for brushing and it needs high pressures to irrigate it with detergent and disinfectant. So this channel needs specific manual irrigation using a syringe, followed by connection in an endoscope

washer-disinfector to a pump that generates significantly higher pressure than is used for any other channel.

The UK first encountered this problem in 2004 in a hospital in Northern Ireland. Dried “stained fluid” was noticed on the floor of a storage cabinet beneath a duodenoscope. It was realised that staff had not known of the existence of the raiser-bridge channel, which was not decontaminated. The **resulting enquiry** into endoscope decontamination, chaired by Dame Deirdre Hine, made many recommendations including that all endoscope channels need to be decontaminated after all procedures, even if they were not used. This has been expanded on in subsequent national guidance, particularly the English Choice Framework for Local Policy & Procedure (CFPP) 01-06 “***Management and decontamination of flexible endoscopes***”. Whilst this should have pre-empted transmission of antibiotic-resistant bacteria via duodenoscopes in the UK, checks that what should happen is reliably occurring may be reassuring.

Peter Hoffman

Trials and tribulations to describe a resistance island…

Antibiotic resistance genes are often located in large genomic islands that contain a whole string of resistance genes. These are usually chromosomally located in ‘hotspots’ for island insertion, but occasionally may be on plasmids. Finding the context of these genes is often fraught with difficulties, even with the advent of whole genome sequencing (WGS). This technology usually relies on breaking the genome into small bits, and then attempting to put the bits back together again from overlaps in the sequences. However, resistance genes are often surrounded by repeat elements that are found in multiple locations in the genome, with the consequence that most assemblies will place the gene by itself, providing no information on its location.

Back in 2010, I attempted to describe a resistance island in an isolate of *Acinetobacter baumannii* that had been sequenced by Roche 454 technology. By doing lots of PCRs and conventional sequencing to link relevant WGS-generated contigs, I described a 14.5 kb ‘AbaR4 type’ island containing $bla_{\text{OXA-23}}$ in a chromosomal insertion hotspot. However, there were concerns raised that the relatively small fragments were not necessarily all joined together in the same island and, even if they were, the island might have been on a plasmid rather than in the chromosomal hotspot.

I needed long reads to join the various elements of the island together definitively and so I joined Oxford Nanopore Technologies **minION Access Programme**. The minION is a tiny sequencer that plugs into a laptop that gives long sequence reads. In a single run I got nearly 600 million bases of sequence with a modal read length of 23,000 bases! Many reads contained $bla_{\text{OXA-23}}$ and were assembled into a massive contig of 252 kb. The region corresponding to the island agreed with my original description, including the insertion in the chromosomal hotspot! Even better, the sequence generated could be placed on a whole genome map of this isolate, and matched!

Now we have seen what it can do, we hope to use the minION to discover the wider context of selected other resistance genes in the near future.

Jane Turton

Changing cephalosporins

Two new beta-lactamase inhibitor combinations have recently been approved by the US FDA, both licensed for complicated urinary and intra-abdominal infections. Review by the European Medicines Agency is pending.

Ceftolozane-tazobactam (Cubist-MSD) protects a new cephalosporin with an old inhibitor. Ceftolozane has 4-8-fold lower MICs than ceftazidime for *P. aeruginosa,* overcoming efflux- and AmpC-mediated resistance. ESBLs hydrolyse ceftolozane but tazobactam inhibits them, restoring susceptibility. Isolates with KPC and metallo-carbapenemases are resistant, whilst the status of Enterobacteriaceae with OXA-48 or copious AmpC remains to be fully defined. Since the most obvious *in vitro* gain is against *P. aeruginosa*, it's unfortunate that the licensing trials didn't include pulmonary infections where this species is prominent. A ventilator pneumonia trial is now underway and evaluation is needed in chronic pseudomonal infections of the lung.

The second new therapy combines ceftazidime, an old cephalosporin, with avibactam, a new inhibitor that protects against ESBLs, AmpC and KPC enzymes. Strains with OXA-48 are susceptible too: OXA-48 doesn't attack ceftazidime and avibactam protects against co-produced ESBLs. Enterobacteriaceae and *P. aeruginosa* with metallo-carbapenemases are resistant.

The advent of these new combinations has led AMRHAI to review its cephalosporin battery. From this Summer, both will be tested against all referred Enterobacteriaceae and *P. aeruginosa*. This will allow us to learn about these new agents *in vitro*, their value for interpretive reading and for therapeutic advice. To create space in our MIC system, we will ultimately stop testing ceftazidime-clavulanate, and unprotected piperacillin.

Until the new combinations are licensed in the UK and to ensure good governance, we will only release results in conjunction with our medical team on a named patient basis. Requests for any drug use on this basis should be sent to the generic AMRHAI email address (**amrhai@phe.gov.uk**), from which a register will be derived.

David Livermore, Robert Hill and Nandini Shetty

How to cap an escalating MIC service?

Antibiotic susceptibility testing (AST) is essential for identifying emerging resistance and for providing *in vitro* data for therapeutic guidance. We use a reference method (agar dilution MIC determination) to be able to adjudicate on discrepant results or those of concern to hospital laboratories, and this methodology is time consuming. Please therefore give due consideration to the investigations you really need and don't ask for MICs if you don't want them. With the advent of screening for carbapenemase producers our AST workloads have increased enormously and need to be managed.

To do this, we will no longer determine MICs on confirmed carbapenemase-producing Enterobacteriaceae (CPE) from rectal / faecal screens (ie gut colonisation). We do this reluctantly because we lose the information that can be inferred from susceptibilities to an extensive panel of antibiotics. We will, of course, continue to

determine MICs for CPE from other sample types, and for all isolates sent in for carbapenemase testing and found PCR-negative.

Looking into the future, we are seeking alternative ways to deliver the MIC service so that we are better able to cope with the rising numbers of multi-resistant bacteria that you find on a weekly basis.

Robert Hill

Launch of our new Bacterial Identification service

We are continuing to merge work streams for the identification of unknown bacteria of MISU and AMRHAI to create a new Bacterial Identification Section (BIDS) within AMRHAI. Staff have been brought together under a single management structure and additional staff recruitment and training is underway. Testing algorithms have been mapped for bacterial identification of unknowns, which means in the near future all unknown isolates received will first be identified by MALDI-ToF MS, and 16S rRNA gene analysis will only be done if reliable species identification is not achieved. Phenotypic testing will be retained for certain groups of organisms where species identification is doubtful or requires additional confirmation. Bacterial identification from normally sterile site clinical samples by 16S rRNA gene analysis will also be provided from within this team.

Julie Logan

Changes to our Resistance Mechanisms services…

In our Winter 2014 Newsletter we reported on how we were working towards faster screening for carbapenemase-producing Enterobacteriaceae. Since then, in collaboration with Matt Ellington (seconded from PHE Cambridge), we have developed and validated a real-time PCR that detects KPC, OXA-48-like, NDM and VIM carbapenemases, as well as having an internal positive control. The assay has been validated by AMRHAI against a panel of 450 isolates with previously defined carbapenem resistance mechanisms and can be run either on the Applied Biosystems® 7500 fast or Rotor-Gene® Q platforms. The assay is now in use as our reference service front-line screen for carbapenemase genes. A multicentre evaluation in the Birmingham, Cambridge, Leeds and Manchester PHE laboratories has shown the assay to be robust and portable. It's already in use at PHE Birmingham, where suspected carbapenemase producers from the West Midlands are tested. In the future we hope that the assay can be deployed to other PHE specialist microbiology laboratories. AMRHAI will then have more time to focus on the national molecular epidemiology (using whole genome sequencing) and investigation of unexplained carbapenem resistance.

In addition, Jackie Findlay has trialled three commercial molecular assays for detecting major carbapenemase families. In summary, the assays offered a reliable means of detecting bacteria producing KPC, OXA-48, NDM or VIM enzymes. Whether your laboratory chooses to use a molecular test, and the subsequent choice of test, will depend on cost (both per isolate and for any proprietary equipment), intended throughput,

preferred gene coverage and ability to fit into local workflows. You can read more about the evaluation **here**.

Katie Hopkins

…and enhanced surveillance of carbapenemase-producing Gram-negative bacteria

We have often stated that we get insufficient patient-level information for many of the bacterial isolates sent to us. To address this for a key group of bacteria, the enhanced surveillance of carbapenemase-producing Gram-negatives commenced in May 2015. Laboratories should use the web-based **Electronic Reporting System** (ERS) to request characterisation of Gram-negative bacteria where expression of an acquired carbapenemase is suspected. The ERS will collect information on patient demographics, submitting laboratory (including specimen details), healthcare setting and risk factors. Some of this information must be provided at the time of isolate referral (core dataset). All other information (enhanced dataset) should be provided within seven days of the isolate being confirmed as a carbapenemase-producer by AMRHAI or by a PHE specialist microbiology laboratory offering deployed service (as Katie writes above). The results of microbiology investigations will be made available via the system in addition to reports issued via the eLab system as at present.

The completion of the core dataset by the sending laboratory will create a record in the ERS. The laboratory is therefore responsible for providing the core dataset at time of isolate referral. However, the laboratory should be supported by the Infection Prevention and Control Team (IPCT) and other NHS trust colleagues in the provision of enhanced surveillance data.

Regular analysis of data captured by the ERS will allow us to identify patient groups that may be more affected by carbapenemase-producing Gram-negative bacteria, monitor changes in the epidemiology of these bacteria and evaluate prevention and control interventions. The value of analyses derived from the enhanced surveillance data will rely on the provision of high-quality data by those referring isolates and populating the enhanced surveillance dataset.

Rachel Freeman
(Field Epidemiology Training Programme Fellow)

Do you use GeneXpert CarbaR ?

AMRHAI is planning to work with Cepheid to evaluate their Remote Xpert cloud-based system to capture and analyse centrally information on CarbaR tests run in selected UK laboratories. This system is an option for GeneXpert users and has potential to contribute to epidemiological monitoring of carbapenemases; it has been used already for TB and influenza. We seek to recruit UK labs who are using the CarbaR test for a six-month survey. We would NOT capture any patient identifiable information, just numbers tested,

numbers positive and which genes were detected where. If you are interested in learning more or taking part please send an email to **me** and Fred Tenover at Cepheid.

Neil Woodford

Proposed changes to Gram-positive serology services

As is the case for the whole of PHE, we are being asked to review the services we offer as part of our core public health function. Looking ahead, as we rationalise our services, we propose discontinuing our Gram positive serology services this Autumn, specifically:
Group A streptococci (anti-streptolysin O and anti-DNase)
Staphylococcus aureus (anti-staphylolysin and anti-nuclease).
Regarding Group A streptococci (GAS), there are various commercial kits available for serological testing. As this is neither a specialised nor a reference test, it is not appropriate for us to be performing the assay routinely. There is also a UK NEQAS scheme for GAS serology testing that laboratories can participate in.
The *S. aureus* assays were developed in-house many years ago and require extensive re-design/re-validation to meet the standards required by ISO15189. We therefore propose to discontinue offering this service and plan to work with our partners in academia to develop a new assay with broader clinical utility and validated to ISO15189 standards.
Before discontinuing either of these services, we will of course inform our customers of likely timescales in advance by letter.

Angela Kearns and Nan Shetty

For *our* safety, use the current versions of our request forms

In recent years, the majority of the RIDDORs in the Bacteriology Reference Department have arisen through misclassification of isolates or material by sending laboratories. As a result, the HSE has issued us various instructions, which include improving communication and follow-up, and also improving and updating our referral forms.

The current versions of the forms have been in use since April 2014 and copies pre-addressed with your details (and an interactive index to help you identify which form to use) are available on request from **limshelpdesk@phe.gov.uk**. Blank forms can also be downloaded directly from our **website**. These forms were not just rebranded with the Public Health England logo but have also undergone other revisions to reflect any changes in the reference services provided and to improve data capture. All forms (H1, H2, H3 and M1) have a safety question in a highlighted box on the form, which should be completed for all submissions. Requests for work on isolates or samples that fall presumptively into ACDP Hazard Group 3 MUST be clearly marked to show the findings of the sending laboratory. Failure to do so may result in unnecessary delays or even specimen rejection, so please raise awareness within your teams of this vital requirement.

We would also like to remind those laboratories that serve more than one hospital to please include the hospital name if different from the sending laboratory. This is particularly important for isolates submitted for investigation of carbapenem resistance as this information can be used by our field epidemiology services colleagues to undertake

enhanced surveillance of confirmed carbapenemase-producing isolates. There is a specific field for this information on our H2 form but if using form H1 for submission of multiple isolates please include this information in the 'additional information' section. Data on patient location at the time of specimen collection will also be recorded via the web-based ESR described above.

Katie Hopkins and Julie Logan

Postcards from Sierra Leone

Between mid-November 2014 and the end of March 2015, seven members of AMRHAI went to Sierra Leone to work in the PHE laboratories providing Ebola testing for treatment centres in Kerry Town, Makeni and Port Loko, staying for five weeks each. Bruno Pichon and Richard Loy were the first to go, helping to set up the laboratory in Port Loko (benches and all!) and then beginning the testing there. Michaela Day and Jacqueline Findlay left just before Christmas to work in Makeni, while I went to Kerry Town. Amy Coward followed in January, and then Kate Martin in February, forming a continuous line of AMRHAI staff in Kerry Town. Amy, Kate and I stayed in 'The Place' beach resort in Tokeh, which was beautiful (perfect white sand beach with perfect weather!), but all the centres had their advantages; in Makeni, staff were able to come and go more freely, and in Port Loko they experienced the wildlife at closer quarters (see right)! We all had a week's training by the Novel and Dangerous Pathogens team at PHE Porton prior to leaving, so we had some idea of what to do! We worked in teams of 12–16, drawn from PHE staff and volunteers from NHS and other laboratories, split into shifts to cover each day from 6 am to 10 pm, seven days a week. We very quickly got used to the smell of chlorine, which was everywhere! We carried out PCR testing from blood and swab samples for the Zaire Ebola virus following inactivation (in an isolator) and extraction of the RNA. Samples were from the treatment centre, the MoD wards (treating healthcare workers) and the community. It was hard work, but very rewarding, especially when we could see the numbers of cases going down, and when we watched recovered patients leaving the treatment centre following negative test results from our laboratories. Some other highlights from our stays were:

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Jane – seeing wild monkeys in the trees, although the snake in specimen reception wasn't such a welcome surprise!

Bruno - the opening of Porto Loko lab and grilled lobster on Tokeh beach

Richard - during set up, being one of the best paid warehouse men in Sierra Leone and spending an afternoon crawling over the top of a flat-bed truck, first in the baking sunshine, followed by pouring rain going through boxes that contained both Port Loko and Makeni's laboratory supplies and throwing probably breakable things over a fence as the fork-lift driver had been banned from the camp.

Michaela - seeing the first Makeni ETC recovery patient sing and dance their way out of the centre to be reunited with their family

Jackie - the seemingly endless line of smiling and waving children from the local village calling out 'Apoto' wherever we went.

Amy - seeing patients get discharged from the Kerry Town ETC and working with scientists from all over the UK, who I hope will be friends for life!
Neil (who didn't go) – seeing all of my Unit's volunteers return safely; seeing the way that other AMRHAI members stepped up to continue services; they all made me very proud !

Jane Turton and the Ebola Volunteers

Staffing and visitors

Since our last issue, AMRHAI has sadly said farewell to Natasha Bundock, Christine Carr, Mahin Khadjavi, Maimuna Kimuli, Christiana Moigboi, Lisa Tokar Lee and Catherine Wiggins. We also said good-bye to Marina Warner who started at Colindale in the mid-1980s as one of the founding members of the Antibiotic Reference Unit.

We are very pleased to be joined by Julie Logan and Sally Langham (formerly of the Microbial Identification Services Unit, MISU) who will work with Jayesh Shah to form our new Bacterial Identification Service (BIDS). Welcome back also to Matt Ellington, who joins us from PHE Cambridge to oversee HPRU projects and WGS deployment.

We extend very warm welcomes to our new starters Oluwafemi Akineremi (from the Oxford HPRU), Eleni Katsis and Samantha Schofield. We welcome Anna Vickers back from maternity leave, and welcome Valia Vasiliki, who is visiting from Greece for 5 months to study VIM-producing Enterobacteriaceae (funded by an award from the HIS).

Much thanks is owed to our various Agency staff (Hayley Dodd, Uma Chandegra, Carina Kulishev, Abdikarin Mire, MacDonald Prest, Mohammed Surti and Lisa Toker Lee) who've provided valuable and much-needed support to cover for Ebola absences and vacancies. Finally, a temporary 'farewell' to Lauren Harwin, now on maternity leave.

AMRHAI senior staff …for when you need advice.

Generic contact details

General enquiries; ***amrhai@phe.gov.uk***	Reference services; placement; visits
Bacteriology Triage; Tel 020-8327-7887	Specimen / result / report queries
Prof. Neil Woodford (Head of Unit) **Neil.woodford@phe.gov.uk**; Tel 020-8327-6511	Resistance mechanisms; R&D opportunities; commercial opportunities (esp. molecular test evaluations)
Dr. Robert Hill **Robert.hill@phe.gov.uk**; Tel 020-8327-7237	Susceptibility testing; interpreting antibiograms; treatment
Mr. Peter Hoffman **Peter.hoffman@phe.gov.uk**; Tel 020-8327-7274	Infection prevention and control; site visits
Dr. Katie Hopkins **Katie.hopkins@phe.gov.uk** ; Tel 020-8327-7061	Resistance mechanisms; inferring mechanisms from antibiograms; commercial opportunities (esp. molecular test evaluations)
Prof. Angela Kearns **Angela.kearns@phe.gov.uk**; Tel 020-8327-7227	Staphylococci; ID & typing; PVL / other toxins; staph/strep serodiagnosis
Prof. David Livermore **david.livermore@phe.gov.uk**; Tel 020-8327-6511	Commercial opportunities (esp. antibiotic evaluations); surveys
Dr Julie Logan **Julie.logan@phe.gov.uk;** Tel 020-8327-6059	Bacterial Identification Service (incl. culture negative clinical specimens)
Dr. Jane Turton **Jane.turton@phe.gov.uk**; Tel 020-8327-7224	Gram-negative typing and serodiagnosis; enterococci
Consultant Microbiologists **Colindalemedmicro@phe.gov.uk**	For medical advice please email or phone 020-8200-4400 and ask for the duty Microbiologist.

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